

[*Cancer Res.*, 57, 246-252 (1997)]

[Lab. of Biochemistry]

**Chemoprevention of 4-Nitroquinoline 1-Oxide-induced Oral Carcinogenesis in Rats by Flavonoids Diosmin and Hesperidin, Each Alone and in Combination.**

Takuji TANAKA, Hiroki MAKITA, Masami OHNISHI, Hideki MORI, Kumiko SATOH, Akira HARA,\*  
Takashi SUMIDA, Keisou FUKUTANI, Tsukasa TANAKA and Hiroshi OGAWA

The modifying effects of the two flavonoids diosmin and hesperidin given during the initiation and postinitiation phases of oral carcinogenesis initiated with 4-nitroquinoline 1-oxide (4-NQO) were investigated in male F344 rats. The compounds were tested alone and in combination. Dietary administration of these compounds significantly decreased the expression of cell proliferation biomarkers (5-bromodeoxyuridine-labeling index and AgNORs) of the nonlesional tongue squamous epithelium, and caused a significant reduction in the frequency of the tongue carcinoma. These findings suggest that diosmin and hesperidin supplementation is effective in inhibiting the development of oral carcinogenesis initiated with 4-NQO.

[*Biochem. J.*, 322, 89-93 (1997)]

[Lab. of Biochemistry]

**Involvement of Two Basic Residues (Lys-270 and Arg-276) of Human Liver 3 $\alpha$ -Hydroxysteroid Dehydrogenase in NADP(H) Binding and Activation by Sulphobromophthalein: Site-directed Mutagenesis and Kinetic Analysis.**

Kazuya MATSUURA, Yoshiyuki TAMADA, Kumiko SATO, Harunori IWASA,  
Gunpei MIWA, Yoshihiro DEYASHIKI and Akira HARA\*

A human liver 3 $\alpha$ -hydroxysteroid dehydrogenase isoenzyme, a member of the aldo-keto reductase family, shows a marked preference for NADP(H) over NAD(H), and is activated by sulphobromophthalein. The mutated enzymes, K270M and R276M, showed increases in the  $K_m$  for NADP<sup>+</sup> of 22- and 290-fold respectively, and attenuated activation by sulphobromophthalein. The results suggest that the two basic residues in the 3 $\alpha$ -hydroxysteroid dehydrogenase isoenzyme play crucial roles in binding both the negatively charged 2'-phosphate group of NADP<sup>+</sup> and the sulphonic groups of sulphobromophthalein.

[*YAKUGAKU ZASSHI*, 117, 167-177 (1997)]

[Lab. of Biochemistry]

**A Sensitive Fluorometric Assay for Dihydrodiol Dehydrogenase.**

Yoshiyuki MIYABE, Kazuya MATSUURA, Toshihiro NAKAYAMA,  
Yoshihiro DEYASHIKI, Isao OHYA and Akira HARA\*

Dihydrodiol dehydrogenase (DD) oxidizes naphthalene dihydrodiol to 1,2-dihydronaphthalene, which is immediately autoxidized to 1,2-naphthoquinone. Here we established a fluorometric assay for the enzyme, which is based on the conversion of 1,2-naphthoquinone to fluorescent compounds by reacting with ethylenediamine. The formed fluorescent compounds were synthetically identified as 6-(2-aminoethylamino)benzo[f]quinoxaline and 2,6- or 3,6-bis(2-aminoethylamino)benzo[f]quinoxaline, which showed the same fluorescence at 550 nm at an excitation wavelength of 420 nm. The method provides a 9000-fold increase in sensitivity over a currently available assay which measures the change in the absorbance of a cofactor, NADPH. Since this method allowed many samples to be assayed simultaneously, we applied it to the analysis of multiple forms of DD, separated by an anion-exchange chromatography, from six human liver specimens.

[*Carcinogenesis*, 18, 761-769 (1997)]

[Lab. of Biochemistry]

**Modulation of N-Methyl-N-amyl nitrosamine-Induced Rat Oesophageal Tumourigenesis by Dietary Feeding of Diosmin and Hesperidine, Both Alone and in Combination.**

Takuji TANAKA, Hiroki MAKITA, Kunihiro KAWABATA, Hideki MORI, Mikio KAKUMOTO, Kumiko SATOH,  
Akira HARA,\* Takashi SUMIDA, Keisou FUKUTANI, Tsukasa TANAKA and Hiroshi OGAWA

The modifying effects of diosmin and hesperidin during the initiation and postinitiation phases of oesophageal carcinogenesis initiated with N-methyl-N-amyl nitrosamine (MNAN) were investigated in male Wistar rats. Feeding of these compounds significantly decreased the expression of cell proliferation biomarkers (BrdU-labelling index, AgNORs and polyamine levels). A number of oesophageal neoplasms developed in rats treated with MNAN alone, and feeding of both compounds caused a significant reduction in the multiplicities of oesophageal carcinoma and papilloma. These findings suggest that diosmin and hesperidin supplementation is effective in inhibiting the development of oesophageal cancer induced by MNAN.